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Use of in vivo-induced antigen technology (IVIAT) for the identification of Streptococcus suis serotype 2 in vivo-induced bacterial protein antigens.

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Abstract

BACKGROUND: Streptococcus suis serotype 2 (SS2) is a zoonotic agent that causes death and disease in both humans and swine. A better understanding of SS2-host molecular interactions is crucial for understanding SS2 pathogenesis and immunology. Conventional genetic and biochemical approaches used to study SS2 virulence factors are unable to take into account the complex and dynamic environmental stimuli associated with the infection process. In this study, in vivo-induced antigen technology (IVIAT), an immunoscreening technique, was used to identify the immunogenic bacterial proteins that are induced or upregulated in vivo during SS2 infection.
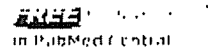
RESULTS: Convalescent-phase sera from pigs infected with SS2 were pooled, adsorbed against in vitro antigens, and used to screen SS2 genomic expression libraries. Upon analysis of the identified proteins, we were able to assign a putative function to 40 of the 48 proteins. These included proteins implicated in cell envelope structure, regulation, molecule synthesis, substance and energy metabolism, transport, translation, and those with unknown functions. The in vivo-induced changes in the expression of 10 of these 40 genes were measured using real-time reverse transcription (RT)-PCR, revealing that the expression of 6 of the 10 genes was upregulated in the in vivo condition. The strain distribution of these 10 genes was analyzed by PCR, and they were found in the most virulent SS2 strains. In addition, protein sequence alignments of the newly identified proteins demonstrate that three are putative virulence-associated proteins.

CONCLUSION: Collectively, our results suggest that these in vivo-induced or upregulated genes may contribute to SS2 disease development. We hypothesize that the identification of factors specifically induced or upregulated during SS2 infection will aid in our understanding of SS2 pathogenesis and may contribute to the control SS2 outbreaks. In addition, the proteins identified using IVIAT may be useful potential vaccine candidates or virulence markers.

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Use of in vivo-induced antigen technology for identification of Escherichia coli O157:H7 proteins expressed during human infection.

John M, Kudva IT, Griffin RW, Dodson AW, McManus B, Krastins B, Sarracino D, Progulske-Fox A, Hillman JD, Handfield M, Tarr PI, Calderwood SB.

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Abstract

Using in vivo-induced antigen technology (IVIAT), a modified immunoscreening technique that circumvents the need for animal models, we directly identified immunogenic Escherichia coli O157:H7 (O157) proteins expressed either specifically during human infection but not during growth under standard laboratory conditions or at significantly higher levels in vivo than in vitro. IVIAT identified 223 O157 proteins expressed during human infection, several of which were unique to this study. These in vivo-induced (ivi) proteins, encoded by ivi genes, mapped to the backbone, O islands (OIs), and pO157. Lack of in vitro expression of O157-specific ivi proteins was confirmed by proteomic analysis of a mid-exponential-phase culture of E. coli O157 grown in LB broth. Because ivi proteins are expressed in response to specific cues during infection and might help pathogens adapt to and counter hostile in vivo environments, those identified in this study are potential targets for drug and vaccine development. Also, such proteins may be exploited as markers of O157 infection in stool specimens.

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Screening of genes expressed in vivo after infection by Vibrio anguillarum M3.

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Abstract
Aims: Genes uniquely expressed in vivo may contribute to the overall pathogenicity of an organism and are likely to serve
as potential targets for the development of new vaccine. This study aims to screen the genes expressed in vivo after Vibrio
anguillarum infection by in vivo-induced antigen technology (IVIAT). Methods and Results: The convalescent-phase sera
were obtained from turbot (Scophthalmus maximus) survived after infection by the virulent V. anguillarum M3. The pooled
sera were thoroughly adsorbed with M3 cells and Escherichia coli BL21 (DE3) cells. A genomic expression library of M3
was constructed and screened for the identification of immunogenic proteins by colony immunoblot analysis with the
adsorbed sera. After three rounds of screening, 19 putative in vivo-induced (ivi) genes were obtained. These ivi genes were
catalogued into four functional groups: regulator/signalling, metabolism, biological process and hypothetical proteins. Three
ivi genes were insertion-mutated, and the growth and 50% lethal dose (LD(50)) of these mutants were evaluated.
Conclusions: The identification of ivi genes in V. anguillarum M3 sheds light on understanding the bacterial pathogenesis
and provides novel targets for the development of new vaccines and diagnostic reagents. Significance and Impact of the
Study: To the best of our knowledge, this is the first report describing in vivo-expressed genes of V. anguillarum using
IVIAT. The screened ivi genes in this study could be new virulent factors and targets for the development of vaccine, which
may have implications for the development of diagnostic regents.

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Identification of in vivo induced protein antigens of Salmonella enterica serovar Typhi during human infection.

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Abstract

During infectious disease episodes, pathogens express distinct subsets of virulence factors which allow them to adapt to different environments. Hence, genes that are expressed or upregulated in vivo are implicated in pathogenesis. We used in vivo induced antigen technology (IVIAT) to identify antigens which are expressed during infection with Salmonella enterica serovar Typhi. We identified 7 in vivo induced (IVI) antigens, which included BcfD (a fimbrial structural subunit), GrxC (a glutaredoxin 3), SapB (an ABC-type transport system), T3663 (an ABC-type uncharacterized transport system), T3816 (a putative rhodanese-related sulfurtransferase), T1497 (a probable TonB-dependent receptor) and T3689 (unknown function). Of the 7 identified antigens, 5 antigens had no cross-immunoreactivity in adsorbed control sera from healthy subjects. These 5 included BcfD, GrxC, SapB, T3663 and T3689. Antigens identified in this study are potential targets for drug and vaccine development and may be utilized as diagnostic agents.

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Identification and immunoprotective analysis of an in vivo-induced Edwardsiella tarda antigen.

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Abstract

Edwardsiella tarda is a severe aquaculture pathogen that can infect many important fish species cultured worldwide. The aim of this study was to evaluate the vaccine potential of an E. tarda antigen, Eta21, which was identified from a pathogenic E. tarda strain via the method of in vivo-induced antigen technology (IVIAT). Eta21 is 510-amino acid in length and shares approximately 58% sequence identity with a putative peptidase of several bacterial species. eta21 was subcloned into Escherichia coli, and recombinant Eta21 was purified as a histidine-tagged protein. When used as a subunit vaccine, purified recombinant Eta21 was effective against lethal E. tarda challenge in a Japanese flounder model. In order to improve the immunoprotective efficacy of Eta21, the chimera AgaV-Eta21 was constructed, which consists of Eta21 fused in-frame to the secretion domain of AgaV, an extracellular beta-agarase. E. coli DH5alpha harboring plasmid pTAET21, which constitutively expresses agaV-eta21, was able to produce and secret AgaV-Eta21 into the extracellular milieu. Vaccination of Japanese flounder with live DH5alpha/pTAET21 elicited immunoprotection that is significantly higher in level than that induced by vaccination with purified recombinant Eta21. Vaccination with DH5alpha/pTAET21 and recombinant Eta21 both induced the production of specific serum antibodies at four to eight weeks post-vaccination. Taken together, these results demonstrate that Eta21, especially that delivered by DH5alpha/pTAET21, is an effective vaccine candidate against E. tarda infection.

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Publication Types, MeSH Terms, Substances, Secondary Source ID

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Genes of periodontopathogens expressed during human disease.

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Abstract

BACKGROUND: Since many bacterial genes are environmentally regulated, the screening for virulence-associated factors using classical genetic and molecular biology approaches can be biased under laboratory growth conditions of a given pathogen, because the required conditions for expression of many virulence factors may not occur during in vitro growth. Thus, technologies have been developed during the past several years to identify genes that are expressed during disease using animal models of human disease. However, animal models are not always truly representative of human disease, and with many pathogens, there is no appropriate animal model.

METHODS: A new technology, in vivo-induced antigen technology (IVIAT) was thus engineered and tested in our laboratory to screen for genes of pathogenic organisms induced specifically in humans, without the use of animal or artificial models of infection. This technology uses pooled sera from patients to probe for genes expressed exclusively in vivo (or ivi, in vivo-induced genes). IVIAT was originally designed for the study of Actinobacillus actinomycetemcomitans pathogenesis, but we have now extended it to other oral pathogens including Porphyromonas gingivalis.

RESULTS: One hundred seventy-one thousand (171,000) clones from P. gingivalis strain W83 were screened and 144 were confirmed positive. Over 300,000 A. actinomycetemcomitans clones were probed, and 116 were confirmed positive using a quantitative blot assay.

CONCLUSION: MAT has proven useful in identifying previously unknown in vivo-induced genes that are likely involved in virulence and are thus excellent candidates for use in diagnostic : and therapeutic strategies, including vaccine design.

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Publication Types, MeSH Terms, Substances, Grant Support

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